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(54) Title: OB RECEPTOR ISOFORMS AND NUCLEIC ACIDS ENCODING THEM (57) Abstract The <i>ob</i> receptor has numerous isoforms resulting from alternative splicing; three novel isoforms, designated c', f, and g are disclosed. The nucleic acids encoding these isoforms are taught. Also part of the invention are vectors containing the nucleic acid encoding the receptors, host cells transformed with these genes, and assays which use the genes or protein isoforms.		

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TITLE OF THE INVENTION**OB RECEPTOR ISOFORMS AND NUCLEIC ACIDS ENCODING THEM****FIELD OF THE INVENTION**

5 This invention relates to *ob* receptor protein isoforms, to DNA and RNA sequences encoding them, and to assays using the receptor isoform proteins.

BACKGROUND OF THE INVENTION

10 Recently the identification of mutations in several genes involved in the onset of obesity in rodents have been identified. Of particular interest are mutations discovered in the peptide hormone, leptin, which is a component of a novel signal transduction pathway that regulates body weight (Zhang *et al.* 1994, *Nature* 372:425-432;
15 Chen *et al.* 1996, *Cell* 84:491-495). Leptin was initially discovered by the positional cloning of the obesity gene, *ob*, in mice. Two different *ob* alleles have been identified: one mutation causes the premature termination of the leptin peptide resulting in a truncated protein, and the other mutation changes the transcriptional activity of
20 the *obesity* (*ob*) gene, resulting in a reduced amount of circulating leptin.

 There is a correlation between a decrease in the levels of biologically active leptin and the overt obese phenotype observed in *ob/ob* mice. Recombinant leptin has been shown to induce weight
25 loss in the *ob/ob* mouse but not in the diabetic phenotype *db/db* mouse (Campfield *et al.* 1995, *Science* 269: 546-549; Halaas *et al.* 1995, *Science* 269: 543-546; Pellymounter *et al.* 1995, *Science* 269:540-543; Rentsch *et al.* 1995, *Biochem. Biophys. Res. Comm.* 214:131-136; and Weigle *et al.* 1995, *J. Clin. Invest.* 96:2065-2070).

30 Although the synthesis of leptin occurs in the adipocyte, its ability to decrease food intake and increase metabolic rate appears to be mediated centrally by the hypothalamus. Injection of recombinant leptin into the third ventricle of the brain elicits a similar response as peripheral administration of leptin.

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Furthermore, the recent cloning of the human receptor for the leptin, the ob-receptor (OB-R), reveals that it is transcribed in the hypothalamus (Tartaglia *et al.* 1995, *Cell* 83:1263-1271; Stephens *et al.* 1995, *Nature* 377: 530-532). In addition, a mutation that
5 results in premature termination of the long-form of the mouse OB-R, which is preferentially expressed in the hypothalamus, appears to be responsible for the obese phenotype of the *db/db* mouse (Lee *et al.* 1996, *Nature* 379:632-635; Chua *et al.* 1996, *Science* 271:994-996; and Chen *et al.* 1996, *Cell* 84:491-495).

10 The OB-R from wild type (lean) rats and from rats having the *fatty* mutation (both heterozygous and homozygous *fa*) have been isolated and sequenced. (Patent Application Serial Nos. _____, Attorney Docket Nos. 19642PV and 19642PV2, filed February 22, 1996 and March 22, 1996, which are hereby
15 incorporated by reference.)

Various isoforms of the OB-Rs have also been identified. These isoforms are due to alternative splicing. For example, in the mouse the a form has 5 amino acids following the Lysine at 889; the b form has 273 amino acids after Lysine 889; the c
20 form has 3 amino acids after Lysine 889; and the d form contains 11 amino acids after Lysine 889.

It would be desirable to be able to further experiment with various isoforms in order to better understand obesity, and to be able to clone and produce novel *ob* receptor isoforms to use in
25 assays for the identification of ligands which may be useful in understanding obesity and for its prevention and treatment.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to novel *ob* receptor isoforms
30 designated c', f and g which are substantially free from associated membrane proteins. It also relates to substantially purified *ob* receptor isoform c', f and g proteins. These isoforms are present in various species, including rat, mouse and human.

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Another aspect of this invention is to nucleic acids which encode OB receptor isoforms c', f or g. The nucleic acid may be any nucleic acid which can encode a protein, such as genomic DNA, cDNA, or any of the various forms of RNA. Preferably, the nucleic acid is cDNA.

This invention also includes vectors containing a OB-R isoform c', f or g gene, host cells containing the vectors, and methods of making substantially pure OB-R isoform c', f or g protein comprising the steps of introducing a vector comprising a OB-R isoform c', f or g gene into a host cell, and cultivating the host cell under appropriate conditions such that OB-R isoform c', f or g is produced. The OB-R isoform c', f or g so produced may be harvested from the host cells in conventional ways.

Yet another aspect of this invention are assays which employ OB-R isoform c', f or g. In these assays, various molecules, suspected of being OB-R isoform c', f or g ligands are contacted with a OB-R isoform c', f or g, and their binding is detected. In this way agonists, antagonists, and ligand mimetics may be identified. A further aspect of this invention are the ligands so indentified.

BRIEF DESCRIPTION OF THE FIGURES

FIGURE 1 is the amino acid sequence of wild type rat OB-R.

FIGURE 2 is the cDNA sequence of wild type rat OB-R.

FIGURE 3 is the cDNA sequence encoding rat isoform.

FIGURE 4 is the cDNA specific for Rat isoform c'.

As used throughout the specification and claims, the following definitions apply:

"Substantially free from associated membrane proteins" means that the receptor protein is not in physical contact with any membrane proteins.

"Substantially purified OB-receptor isoform c', f or g" means that the protein isoform is at least 90% and preferably at least 95% pure.

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"Wild type" means that the gene or protein is substantially the same as that found in an animal which is not considered to have a mutation for that gene or protein.

"fa" means that the gene or protein is substantially the same as that found in a rat homologous for the *fatty* mutation.

"Substantially the same" when referring to a nucleic acid or amino acid sequence means either it is the same as the reference sequence, or if not exactly the same, contains changes which do not affect its biological activity or function.

10

It has been surprisingly found, in accordance with this invention that the OB-R exists in a large variety of isoforms, including three novel ones, form c', f and g. These isoforms apply to all species, but for convenience, throughout the specification and claims, numberings of amino acids and nucleotides will use the rat wild type sequences (FIGURES 1 and 2) as a reference. However, it is to be understood that this invention is not limited to rat wild type proteins and nucleic acids and specifically includes rat (wild type and *fatty*), mouse, and human OB-R isoform c', f and g proteins and nucleic acids.

20

OB-R isoform f differs from wild type protein in that after the Lysine at position 889 (referring to the rat sequence in FIGURE 1), there are six amino acids, ending at an Asparagine residue at position 895. In the cDNA, the codons are then followed by a Stop codon. One cDNA for rat isoform f is shown in FIGURE 3; this invention specifically includes all various cDNAs encoding an isoform f protein. The superscripted numbers refer to protein position numbers.

25

Lys⁸⁸⁹ Iso⁸⁹⁰ Met⁸⁹¹ Pro⁸⁹² Gly⁸⁹³ Arg⁸⁹⁴ Asn⁸⁹⁵

In the human isoform f, Lysine 891 corresponds to the rat Lysine 889, the same six amino acids follow Lysine 889.

In a particularly preferred embodiment of this invention, the OB-R isoform f is from rat origin.

30

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OB-R isoform g differs from the wild type in that it is much shorter than the wild type sequence. The following eighteen amino acids are found at the beginning of the protein with the superscript numbers indicating their position. The Arginine at position 18 is spliced to a large fragment of the wild type molecule, beginning at the Proline at position 166 (in both mouse and human). This isoform then extends for the remainder of the wild type molecule.

Met¹ Phe² Gln³ Thr⁴ Pro⁵ Arg⁶ Ile⁷ Val⁸ Pro⁹ Gly¹⁰
His¹¹ Lys¹² Asp¹³ Leu¹⁴ Ile¹⁵ Ser¹⁶ Lys¹⁷ Arg¹⁸ Pro¹⁶⁶...

After Pro¹⁶⁶, the remainder of the protein may be the same as wild type, or, alternatively it could also contain another isoform variation, such as isoform a, b, c, d, e, or f.

A particularly preferred embodiment is the rat isoform g.

OB-R isoform c' is similar to the OB-R isoform c which was previously described [Lee *et al.*, *Nature* 379: 632-635]. After Lysine at position 889, it only has three amino acids, Val⁸⁹⁰ Thr⁸⁹¹ Phe⁸⁹² Stop. As can be seen, isoform c' differs from isoform c in that the final amino acid is phenylalanine rather than valine found in isoform c. Further, there are untranslated sequences in the DNA encoding isoform c' which do not appear to be present in isoform c. The cDNA encoding the rat isoform c' is given in FIGURE 4. In humans, the Val, Thr, Phe follow Lysine 891.

One aspect of this invention is the molecular cloning of these various isoforms of OB-R. The wild type and *fa* receptor proteins contain an extracellular, a transmembrane domain. In the rat, the extracellular domain extends from amino acids 1-830; the transmembrane domain is from amino acids 839-860; and the cytoplasmic domain is from amino acids 860-1162. Similar domains have been identified for the mouse and human proteins. This

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invention also includes isoform c', f and g proteins which lack one or more of these domains. Such deleted proteins are useful in assays for identifying ligands and their binding activity.

5 In the rat wild type protein, amino acids 1-28 form a signal sequence; thus the mature proteins extend from amino acids 28-1162. The mature protein isoforms form yet another aspect of this invention. This differs somewhat from the signal sequence of 1-22 reported for mouse and human OB-R; the mature mouse and human isoforms form yet another aspect of this invention.

10 The OB-R isoform c', f or g gene can be introduced into virtually any host cell using known vectors. Preferred host cells include *E. coli* as well as mammalian and yeast cell lines.

One of ordinary skill in the art is able to choose a known vector which is appropriate for a given host cell; generally
15 plasmids or viral vectors are preferred. The OB-R isoform c', f or g gene may be present in the vector in its native form, or it may be under the control of a heterologous promoter, and if desired, one or more enhancers, or other sequences known to regulate transcription or translation. The host cell containing the OB-R isoform c', f or g
20 gene is cultured, and the OB-R isoform c', f or g gene is expressed. After a suitable period of time the OB-R c', f or g isoform protein may be harvested from the cell using conventional separation techniques.

A further aspect of this invention is the use of an OB-R
25 c', f or g isoform in assays to identify OB-R c', f or g isoform ligands. A ligand binds to the OB-R isoform receptor, and *in vivo* may or may not result in an activation of the receptor. Ligands may be agonists of the receptor (i.e. stimulate its activity), antagonists (inhibit its activity) or they may bind with little or no effect upon the
30 receptor activity.

In an assay for ligands, an OB-R isoform of this invention is exposed to a putative ligand, and the amount of binding is measured. The amount of binding may be measured in many ways; for example, a ligand or the OB-R isoform being investigated

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may be labeled with a conventional label (such as a radioactive or fluorescent label) and then put in contact with the OB-R isoform under binding conditions. After a suitable time, the unbound ligand is separated from the OB-R isoform and the amount of ligand which has bound can be measured. This can be performed with any of the OB-R isoforms of this invention; alternatively the amount of binding of the various isoforms can be compared. In a competitive assay, both the putative ligand and a known ligand are present, and the amount of binding of the putative ligand is compared to the amount of binding to a known ligand. Alternatively, the putative ligand's ability to displace previously bound known ligand (or vice-versa) may be measured. In yet other embodiments, the assay may be a heterogeneous one, where the OB-R isoform may be bound to a surface, and contacted with putative ligands. Detection of binding may be by a variety of methods, including labelling, reaction with antibodies, and chromophores.

In another assay, the OB-R isoforms of this invention may be used in a "trans" activation assay. Such assays are described in U.S. Application Serial No. _____, Attorney Docket No. 19686PV, which was filed on April 22, 1996 and which is hereby incorporated by reference. In this assay, a cell which expresses an OB-R isoform of this invention (either naturally or through recombinant means) is transfected with a reporter gene construct comprising a minimal promoter, a leptin activation element and a reporter gene. Transcription of the reporter gene is dependant upon activation of the leptin activation element. Binding of a ligand to the receptor isoform activates the leptin activation element, which then allows transcription of the reporter gene.

The following non-limiting Examples are presented to better illustrate the invention.

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EXAMPLE 1

Preparation of mRNA and cDNA from rat tissues

Tissues were collected from lean and *fal/fa* Zucker rats
5 and snap frozen in liquid nitrogen. The tissues collected included:
hypothalamus, pituitary, lung, liver, kidney, heart, adrenal glands,
smooth muscle, skeletal muscle, and adipose tissue. The tissues were
homogenized with a Brinkmann Polytron homogenizer in the
presence of guanadinium isothiocyanate. mRNA was prepared from
10 hypothalamus, lung, and kidney according to the instructions
provided with the messenger RNA isolation kit (Stratagene, La Jolla,
CA). cDNA was prepared from approximately 2 µg of mRNA with
the SuperScript™ choice system (Gibco/BRL Gaithersburg, MD).
The first strand cDNA synthesis was primed using 1 µg of
15 oligo(dT)₁₂₋₁₈ primer and 25 ng of random hexamers per reaction.
Second strand cDNA synthesis was performed according to the
manufacturer's instructions. The quality of the cDNA was assessed
by labeling an aliquot (1/10th) of the second strand reaction with
approximately 1 µCi of [α -³²P]dCTP (3000 Ci/mmol). The labeled
20 products were separated on an agarose gel and detected by
autoradiography.

EXAMPLE 2

Preparation of a hypothalamic cDNA library

Approximately 3.6 µg of phosphorylated *Bst* XI adapters
(Invitrogen, San Diego, CA) were ligated to approximately 3 µg of
cDNA prepared as described in Example 1. The ligation mix was
then diluted and size-fractionated on a cDNA sizing column
30 (Gibco/BRL Gaithersburg, MD). Drops from the column were
collected and the eluted volume from the column was determined.
An aliquot from each fraction was analyzed on an agarose gel.
Fractions containing cDNA of greater than or equal to 1 kb were
pooled and precipitated. The size-fractionated cDNA with the *Bst* XI
35 adapters was ligated into the prokaryotic vector pcDNA II

(Invitrogen, San Diego, CA). The vector (4 μ g) was prepared for ligation by first cutting with the restriction endonuclease *Bst* XI, gel purifying the linearized vector, and then dephosphorylating the ends with calf intestinal phosphatase (Gibco/BRL, Gaithersburg, MD).

according to the manufacturers instructions. The ligation contained approximately 10-20 ng of cDNA and approximately 100 ng of vector and was incubated overnight at 14°C. The ligation was transformed into 1 ml of XL-2 Blue Ultracompetent cells (Stratagene, La Jolla, CA) according to the manufacture's instructions. The transformed cells were spread on 133 mm Colony/Plaque Screen filters (Dupont/NEN, Boston, MA), plated at a density of 30,000 to 60,000 colonies per plate on Luria Broth agar plates containing 100 µg/ml Ampicillin (Sigma, St. Louis, MO).

EXAMPLE 3

Screening a hypothalamic cDNA library

Colonies on filters were replica plated onto a second filter set. The master filter was stored at 4°C for subsequent isolation of regions containing colonies that gave a positive hybridization signal. The replica filters were grown for several hours at 37°C until colonies were visible and then processed for in situ hybridization of colonies according to established procedures (Maniatis, et al. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Publications, Cold Spring Harbor, NY, which is hereby incorporated by reference). A Stratalinker (Stratagene, La Jolla, CA) was used to crosslink the DNA to the filter. The filters were washed at 55°C for 2 hours in 2x SSC and 0.5% SDS to remove bacterial debris. Eight to ten filters were then placed in a heat sealable bag (Kapak, Minneapolis, MN) containing 15-20 ml of 1x hybridization solution (Gibco/BRL, Gaithersburg, MD) containing 50% formamide and incubated for 1 hour at 42°C. The filters were hybridized overnight with greater than 1,000,000 cpm/ml of the radiolabeled probe described below in 1x

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hybridization buffer (Gibco/BRL, Gaithersburg, MD) containing 50% formamide at 42°C. The probe, a 2.2 kb fragment encoding the extracellular portion of the Ob-R was labeled by random priming with [alpha ³²P]dCTP (3000 Ci/mmol, Amersham, Arlington Heights, IL) using redi-prime (Amersham, Arlington Heights, IL). The probe was purified from unincorporated nucleotides using a Probequant G-50 spin column (Pharmacia Biotech, Piscataway, NJ). Filters were washed two times with 0.1x SSC 0.1% SDS at 60°C for 30 min and then subjected to autoradiography. Individual regions containing hybridization positive colonies were lined up with the autoradiogram of the hybridized filter. These were excised from the master filter, and placed into 0.5 ml Luria broth plus 20% glycerol. Each positive was replated at a density of approximate 50-200 colonies per 100 by 15 mm plate and screened by hybridization as previously described. Individual positive colonies were picked and plasmid DNA was prepared from an overnight culture using a Wizard kit (Promega, Madison, WI).

EXAMPLE 4

20

Amplification of Lean Rat OB-receptor cDNA using PCR

To provide for a probe to screen the hypothalamic cDNA library, the rat OB receptor was initially obtained by PCR using degenerate primers based on the mouse and human OB-receptor amino acid sequences. A set of oligonucleotide primers, were designed to regions with low codon degeneracy. The pairing of the forward primers ROBR 2 (5'-CAY TGG GAR TTY CTI TAY GT-3') and ROBR 3 (5'-GAR TGY TGG ATG AAY GG-3') corresponding to mouse amino acid sequences HWEFLYV and ECWMKG, with reverse primers ROBR 6 (5'-ATC CAC ATI GTR TAI CC-3'), ROBR 7 (5'-CTC CAR TTR CTC CAR TAI CC-3'), ROBR 8 (5'-ACY TTR CTC ATI GGC CA-3') and ROBR 9 (5'-CCA YTT CAT ICC RTC RTC-3') representing mouse amino acids, GYTMWI, VYWSNWS, WPMSKV, and DDGMKW provided good yields of the appropriately sized products. The fragments of interest

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were amplified as long polymerase chain reaction (PCR) products by a modifying the method of Barnes (1994, *Proc. Natl. Acad. Sci.* 91:2216-2220, which is hereby incorporated by reference). In order to obtain the required long PCR fragments, Taq Extender (Stratagene, La Jolla CA) and the Expand Long Template PCR System (Boehringer Mannheim, Indianapolis, IN) were used in combination. The standard PCR reaction mix, in a final volume of 20 μ l, contained 5 ng of template (lean rat cDNA), 100 ng of primers, 500 μ M dNTPs, 1 X Buffer 3 from the Expand kit, 0.1 μ l each of Taq Polymerase and Taq Expander. Reactants were assembled in thin walled reaction tubes. The amplification protocol was: 1 cycle of 92°C for 30 sec., followed by 32 cycles at 92°C for 30 sec., 45°C for 1 min. and 68°C for 3 min. using a Perkin-Elmer (Norwalk, CT) 9600 Thermal Cycler.

This strategy produced a series of PCR products with the largest being approximately 2.2 Kbp amplified from primers ROBR 2 and ROBR 9. These products were subcloned for DNA sequence analysis as described below. The insert was excised from the cloning vector with the restriction endonuclease *Eco* RI, and fragments were separated from the vector by agarose gel electrophoresis. The fragments were eluted from the gel using a Prep-A-Gene kit (BioRad, Richmond CA) according to the manufacturer's instructions and radiolabeled as described above.

EXAMPLE 5

Subcloning of PCR products

PCR products of the appropriate size were prepared for subcloning by separation on an agarose gel, excising the band, and extracting the DNA using Prep-A-Gene (BioRad, Richmond, CA). PCR products were ligated into pCRTMII (Invitrogen, San Diego, CA) according to the instructions provided by the manufacturer. The ligation was transformed into INVaF' cells and plated on Luria-Bertani plates containing 100 μ g/ml ampicillin and X-Gal (32 μ l of

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50 mg/ml X-Gal (Promega, Madison, WI). White colonies were picked and grown overnight in Luria-Bertani broth plus 100 µg/ml ampicillin. Plasmid DNAs were prepared using the Wizard miniprep kit (Promega, Madison, WI). Inserts were analyzed by digesting the
5 plasmid DNA with EcoRI and separating the restriction endonuclease digestion products on an agarose gel.

Plasmid DNA was prepared for DNA sequencing by ethanol precipitation of Wizard miniprep plasmid DNA and resuspending in water to achieve a final DNA concentration of 100
10 µg/ml. DNA sequence analysis was performed using the ABI PRISM™ dye terminator cycle sequencing ready reaction kit with AmpliTaq DNA polymerase, FS. The initial DNA sequence analysis was performed with M13 forward and reverse primers, subsequently
primers based on the rat OB-R sequence were utilized. Following
15 amplification in a Perkin-Elmer 9600, the extension products were purified and analyzed on an ABI PRISM 377 automated sequencer (Perkin Elmer, Norwalk, CT). DNA sequence data was analyzed with the Sequencher program.

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WHAT IS CLAIMED IS:

1. *Ob*-receptor (OB-R) isoform c', f or g,
substantially free from associated proteins.
- 5 2. An OB-R isoform according to Claim 1 which is
substantially pure.
3. An OB-R isoform according to Claim 1 which is a
10 c' isoform.
4. An OB-R isoform according to Claim 1 which is
an f isoform.
- 15 5. An OB-R isoform according to Claim 1 which is a
g isoform.
6. An OB-R isoform according to Claim 1 which is
from a rat.
- 20 7. An OB-R isoform according to Claim 6 which is
from a wild-type rat.
8. An OB-R isoform according to Claim 6 which is
25 from a *fatty* rat.
9. An OB-R isoform according to Claim 3 which is
human.
- 30 10. An OB-R isoform according to Claim 4 which is
human.
11. An OB-R isoform according to Claim 5 which is
human.

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12. An OB-R isoform according to Claim 3 which is
from a mouse.
13. An OB-R isoform according to Claim 4 which is
5 from a mouse.
14. An OB-R isoform according to Claim 5 which is
from a mouse.
- 10 15. A nucleic acid encoding an OB-R of Claim 1.
16. A nucleic acid according to Claim 15 which is a
cDNA.
- 15 17. A vector comprising a nucleic acid which encodes
an OB-R of Claim 1.
18. A vector according to Claim 17 which is a
plasmid.
- 20 19. A host cell containing a vector according to Claim
17.
20. A host cell according to Claim 19 which is *E. coli*,
25 a mammalian cell, or a yeast cell.
21. An assay to determine if a putative ligand binds to
an OB-R isoform c', f or g comprising: contacting the putative ligand
with an OB-R isoform c', f or g, and determining if binding has
30 occurred.

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22. An assay according to Claim 17 wherein the
ligand is labeled.

23. An assay to determine if a putative ligand binds to
5 an OB-R isoform c', f or g which is a trans-activation assay.

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1 MTCQKFYVVL LHWEFLYVIT ALNLAYPTSP WRKLFCA PP STTDDSF LSP
51 AGVPNNNTSSL KGASEALVEA KFNSTGIYVS ELSKTIFHCC FGNEQGQNC S
101 ALTGNTGKT LASVVKPLVF ROLGVNWDIE CWMKGD LTLF ICHMEPL LKN
151 PFKNYDSKVH LLYDLPEVID DLPLPLKDS FQTVQCNC SV RECECHVPVP
201 RAKVNYALLM YLEITSAGVS FQSP LMSLOP MLVVKPDPPL GLRMEVTDDG
251 NLKISWDSQT KAPFPLOQYV KYLENSTIVR EAAEIVSDTS LLVDSVLP GS
301 SYEVQVRSKR LDGSGVWSDW SLPQLFTTQD VMYFPPKILT SVGSNASFCC
351 IYKNENQ TIS SKQIVMMNL AEKIPETQYN TVSDHISKVT FSNLKATRPR
401 GKFTYDAVYC CNEQACHRY AELYVIDVNI NISCETDGYL TKMTCRWSPS
451 TIQSLVGSTV QLRVHRRSLY CPDNPSIRPT SELKNCVLQT DGFYECVFQP
501 IFLLSGYTMW IRINHSLSL DSPPTCVLPD SVVKPLPPSN VKAEITINTG
551 LLKVSWEKPV FPENNLQFQI RYGLNGKEIQ WKTHEVFDAK SKSASLPVSD

FIG. 1A

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601 LCAVYVVQVR CRRLDGLGYW SNWSSPAYTL VMDVKVPMRG PEFWRIMDGD
651 ITKKERNVTI LMKPLMKNDL LCSVRRYVVK HRTAHNGTWS QDVGNQTNLT
701 FLWAESAHTV TVLAINSIGA SLVNFNLTF S WPM SKVNAVQ SL SAYPLSS S
751 CVILSWTLSP NDYSLLYLVI EWKNLNDDDG MKWLRIPSNV NKYYIHDNFI
801 PIEKYQFSLY PVFMEGVGKP KIINGFTKDD IAKQQNDAGL YVIVPIIIS S
851 CVLLLGTLII SHQRMKKLFW DDVNPKNCS WAQGLNFQKP ETFEHLFTKH
901 AESVIFGPLL LEPEPVSEEI SVDTAWKNKD EMVPAAMVSL LLTPDSTRG
951 SICISDQCNS ANFSGAQSTQ GTCEDQC SQ PSVKYATLVS NVKTVETDEE
1001 QGAIHSSVSQ CIARKHSPLR QSFSSNSWEI EAQAFFLLSD HPPNVISPQL
1051 SFSGLDELLE LEGNFPEENH GEKSVYYLGV SSGNKNRENDM LLTDEAGVLC
1101 PFPACHCLFSD IRILQESC SH FVENNLNLGT SGKNFVPYMP QFQSCSTHSH
1151 KIENKMC DL TV

FIG. 1B

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1 TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT
51 ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAAAAT TCTATGTGCT
101 TTTGTTACAC TGGGAATTC TGTATGTGAT AACTGCACCT AACCTGGCCT
151 ATCCAACCTC TCCCTGGAGA TTAAAGCTGT TTTGTGGGCC ACCGAGTACA
201 ACTGATGACT CCTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC
251 TTTGAAGGGG GCTTCTGAAG CACTTGTGA AGCTAAATTT AATTCAACTG
301 GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG
351 AATGAGCAAG GTCAAAACCTG CTCCGCACTC ACAGGCAACA CTGAAGGGAA
401 GACGCTGGCT TCAGTGGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA
451 ACTGGGACAT AGAGTGCTGG ATGAAAAGGG ACTTGACATT ATTCATCTGT
501 CATATGGAAC CATTACTTAA GAACCCCTTC AGAATTATG ACTCTAAGGT
551 TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTTG CCTCTGCCCC
601 CACTGAAAGA CAGCTTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCTGGGAA
651 TGCGAATGTC ATGTACCAGT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT

FIG. 2A

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701 GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTCAG TCACCTCTAA
751 TGTCACTGCA GCCCATGCTT GTTGTGAAGC CCGATCCACC GCTGGGTTTG
801 CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTTCAT GGGACAGCCA
851 AACAAAAGCA CCATTTCAC TTCAATATCA GGTGAAATAT TTAGAGAAAT
901 CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG
951 GTAGACAGCG TGCTTCCTGG GTCTTCATAC GAGGTCCAGG TGAGGAGCAA
1001 GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTA CCTCAACTCT
1051 TTACCACACA AGATGTCATG TATTTTCCAC CCAAAATTCT GACGAGTGTT
1101 GGATCCAATG CTTCCTTTTG CTGCATCTAC AAAAATGAGA ACCAGACTAT
1151 CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG
1201 AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTTCTCC
1251 AACCTGAAAG CCACCAGACC TCGAGGGAAG TTACCTATG ATGCAGTGTA
1301 CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA

FIG. 2B

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1351 TCGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTTAACTAAA
1401 ATGACTTGCA GATGGTCACC CAGCACAAATC CAATCACTAG TGGGAAGCAC
1451 TGTGCAGTTG AGGTATCACA GGCGCAGCCT GTACTGTCCC GATAATCCAT
1501 CTATTTCGTCC TACATCAGAG CTCAAAAACT GCGTCTTACA GACAGATGGC
1551 TTTTATGAAT GTGTTTTCCA GCCAATCTTT CTATTATCTG GCTATACAAT
1601 GTGGATCAGG ATCAACCATT CTTAGGTTT ACTTGACTCT CCACCAACGT
1651 GTGTCCCTTC TGACTCCGTA GTAAAACCAC TACCTCCATC TAATGTAAAA
1701 GCAGAGATTA CTATAAACAC TGGATTATTG AAAGTATCTT GGGAAAAGCC
1751 AGTCTTTCCA GAGAAATAACC TTCAGTTCCA GATTCGATAT GGCTTAAATG
1801 GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA
1851 TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT
1901 TCGCTGCCGG CGGTGGATG GACTAGGTA TTGGAGTAAT TGGAGCAGTC
1951 CAGCCTACAC TCTTGTTCATG GATGTAAAAG TTCCATATGAG AGGGCCTGAA
2001 TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC

FIG. 2C

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2051 CTTGCTTTGG AAGCCACTGA TGAAAAATGA CTCACTGTGT AGTGTGAGGA
2101 GGTATGTGGT GAAGCATCGT ACTGCCACACA ATGGACATG GTCACAAGAT
2151 GTGGGAAATC AGACCAATCT CACTTTCCTG TGGGCAGAAT CAGCACACAC
2201 TGTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA
2251 ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC
2301 AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC
2351 ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA
2401 ATGATGATGA TGGAAATGAAG TGGCTTAGAA TCCCTTCGAA TGTAAACAAG
2451 TATTATATCC ATGATAATTT TATTCCTATC GAGAAATATC AGTTTAGTCT
2501 TTACCCAGTA TTTATGGAAG GAGTTGGAAA ACCAAAGATA ATTAATGGTT
2551 TCACCAAAGA TGATATCGCC AAACAGCAA AATGATGCAGG GCTGTATGTC
2601 ATTGTACCGA TAATTATTTC CTCTTGTCGTC CTGCTGCTCG GAACACTGTT
2651 AATTTCACAC CAGAGAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC

FIG. 2D

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2701 CCAAGAATTG TTCCTGGGCA CAAGGACTTA ATTTCCAAA GCCTGAAACA
2751 TTGAGCATC TTTTACCAG GCATGCAGAA TCAGTGATAT TTGGTCCCTCT
2801 TCTTCTGGAG CCTGAACCAG TTTCAGAAGA AATCAGTGTC GATACAGCTT
2851 GGAAAAATAA AGATGAGATG GTACCAGCAG CTATGGTCTC ACTTCTTTTG
2901 ACCACTCCAG ATTCCACAAG GGGTTCTATT TGTATCAGTG ACCAGTGTA
2951 CAGTGCTAAC TTCTCTGGGG CTCAGAGCAC CCAGGGAACC TGTGAGGATG
3001 AGTGTGAGAG TCAACCCCTCA GTTAAATATG CAACGCTGGT CAGCAACGTG
3051 AAAACAGTGG AACTGATGA AGAGCAAGGG GCTATACATA GTTCTGTCAG
3101 CCAGTGCATC GCCAGGAAAC ATTCCCCACT GAGACAGTCT TTTTCTAGCA
3151 ACTCCTGGGA GATAGAGGCC CAGGCATTTT TCCTTTTATC AGATCATCCA

FIG. 2E

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3201 CCCAATGTGA TTTCACCACA ACTTTCATTTC TCAGGGTTGG ATGAGCTTTT
3251 GGAAC TGGAG GGA AATTTTC CTGAAGAAAA TCACGGGGAA AAATCTGTGT
3301 ATTATCTAGG AGTCTCCTCA GGAAACAAAA GAGAGAATGA TATGCTTTTG
3351 ACTGATGAGG CAGGGGTATT GTGCCCCATTCCAGCTCACT GTCTGTTCAG
3401 TGACATCAGA ATCCTCCAGG AGAGTTGTTT ACACTTTGTA GAAAAATAATT
3451 TGAATTTAGG GACCTCTGGT AAGAACTTTG TACCTTACAT GCCCCAGTTT
3501 CAATCCTGTT CCACTCACAG TCATAAGATA ATAGAAAAATA AGATGTGTGA
3551 CTTAACTGTG TAATCTTGTG CAAAAACTTC CAGGTTCCAT TCCAGTAGAG
3601 TGTGTCATGT ATAATATGTT CTTTATAGT TGTGGGTGGG AGAGAAAGCC

FIG. 2F

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1 TGGGGCAATT GGGCTGACCT TTCTTATGCT GGGATGTGCC TTGGAGGACT
51 ATGGGTGTCT ATCTCTGAAG TAAGATGACG TGTCAAGAAAT TCTATGTGGT
101 TTTGTTACAC TGGGAATTTC TGTATGTGAT AACTGCACTT AACCTGGCCT
151 ATCCAACCTC TCCCTGGAGA TTAAAGCTGT TTTGTGCGCC ACCGAGTACA
201 ACTGATGACT CCTTCTCTC TCCTGCTGGA GTCCCAAACA ATACTTCGTC
251 TTTGAAGGGG GCTTCTGAAG CACTTGTTGA AGCTAAATT AATTCAACTG
301 GTATCTACGT TTCTGAGTTA TCCAAAACCA TTTTCCACTG TTGCTTTGGG
351 AATGAGCAAG GTCAAAACTG CTCCGCACTC ACAGGCAACA CTGAAGGAA
401 GACGCTGGCT TCAGTGGTGA AGCCTTTAGT TTTCCGCCAA CTAGGTGTAA
451 ACTGGGACAT AGAGTGCTGG ATGAAAGGGG ACTTGACATT ATTCATCTGT
501 CATATGGAAC CATTA CTAA GAACCCCTTC AAGAAATTATG ACTCTAAGGT
551 TCACCTTTTA TATGATCTGC CTGAAGTTAT AGATGATTG CCTCTGCCCC
601 CACTGAAAGA CAGCTTTCAG ACTGTCCAGT GCAACTGCAG TGTTCGGGAA

FIG. 3A

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651 TCGGAATGTC ATGTACCAGT ACCCAGAGCC AAAGTCAACT ACGCTCTTCT
701 GATGTATTTA GAAATCACAT CTGCTGGTGT GAGTTTTCAG TCACCTCTAA
751 TGTCAC TGCA GCCCATGCTT GTTGTGAAGC CCGATCCACC GCTGGGTTTG
801 CGTATGGAAG TCACAGATGA TGGTAATTTA AAGATTTCAT GGGACAGCCA
851 AACAAAAGCA CCATTTCAC TTCAATATCA GTGAAATAT TTAGAGAATT
901 CTACAATCGT AAGAGAGGCT GCTGAAATCG TCTCGGATAC ATCTCTGCTG
951 GTAGACAGCG TGCTTCCTGG GTCTTCATAC GAGTCCAGG TGAGGAGCAA
1001 GAGACTGGAT GGCTCAGGAG TCTGGAGTGA CTGGAGTTA CCTCACTCT
1051 TTACCACACA AGATGTCATG TATTTTCCAC CCAAATTTCT GACGAGTGTT
1101 GGATCCAATG CTTCCCTTTG CTGCATCTAC AAAAATGAGA ACCAGACTAT
1151 CTCCTCAAAA CAAATAGTTT GGTGGATGAA TCTAGCCGAG AAGATCCCCG
1201 AGACACAGTA CAACACTGTG AGTGACCACA TTAGCAAAGT CACTTCTCC
1251 AACCTGAAAG CCACCAGACC TCGAGGGAAG TTTACCTATG ATGCAGTGTA

FIG. 3B

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1301 CTGCTGCAAT GAGCAGGCAT GCCATCACCG CTACGCTGAA TTATATGTGA
1351 TCGATGTCAA TATCAATATA TCATGTGAAA CTGACGGGTA CTTAACTAAA
1401 ATGACTTGCA GATGGTCACC CAGCACAATC CAATCACTAG TGGGAAGCAC
1451 TGTGCAGTTG AGGTATCACA GGCAGCCT GTACTGTCCC GATAATCCAT
1501 CTATTCTGTC TACATCAGAG CTCAAAACT GCGTCTTACA GACAGATGGC
1551 TTTTATGAAT GTGTTTTCCA GCCAATCTTT CTATTATCTG GCTATACAAT
1601 GTGGATCAGG ATCAACCATT CTTAGGTTT ACTTGACTCT CCACCAACGT
1651 GTGTCCTTCC TGA CTCCGTA GTAAAACCAC TACCTCCATC TAATGTAAAA
1701 GCAGAGATTA CTATAAACAC TGGATTATTG AAAGTATCTT GGGAAAAGCC
1751 AGTCTTTCCA GAGAAATAACC TTCAGTTCCA GATTCGATAT GGCTTAAATG
1801 GAAAAGAAAT ACAATGGAAG ACACACGAGG TATTCGATGC AAAATCAAAA
1851 TCGGCCAGCC TGCCAGTGTC AGATCTCTGT GCGGTCTATG TGGTACAGGT

FIG. 3C

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1901 TCGCTGCCGG CGGTTGGATG GACTAGGGTA TTGGAGTAAT TGGAGCAGTC
1951 CAGCCTACAC TCTTGTCATG GATGTAAAAG TTCCCTATGAG AGGCCCTGAA
2001 TTCTGGAGAA TAATGGATGG GGATATTACT AAAAAGGAGA GAAATGTCAC
2051 CTTGCTTTGG AAGCCACTGA TGAAAAATGA CTCACTGTGT AGTGTGAGGA
2101 GGTATGTGGT GAAGCATCGT ACTGCCACACA ATGGGACATG GTCACAAGAT
2151 GTGGGAAATC AGACCAATCT CACTTTCCCTG TGGGCAGAAT CAGCACACAC
2201 TGTTACAGTT CTGGCCATCA ATTCCATCGG TGCCTCCCTT GTGAATTTTA
2251 ACCTTACGTT CTCATGGCCC ATGAGTAAAG TGAATGCTGT GCAGTCACTC
2301 AGTGCTTATC CCCTGAGCAG CAGCTGCGTC ATCCTTTCCT GGACACTGTC
2351 ACCTAATGAT TATAGTCTGT TATATCTGGT TATTGAATGG AAGAACCTTA
2401 ATGATGATGA TGGAATGAAG TGGCTTAGAA TCCCTTCGAA TGTTAAACAAG
2451 TATTATATCC ATGATAAATT TATTCCATC GAGAAATATC AGTTTAGTCT

FIG. 3D

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2501 TTACCCAGTA TTTATGGAAG GAGTTGAAA ACCAAAGATA ATTAATGGTT
2551 TCACCAAAGA TGATATCGCC AACACGAAA ATGATGCAGG GCTGTATGTC
2601 ATTGTACCGA TAAATTATTC CTCTTGTGTC CTGCTGCTCG GAACACTGTT
2651 AATTTCACAC CAGAGAAATGA AAAAGTTGTT TTGGGACGAT GTTCCAAACC
2701 CCAAGAAATG TTCCTGGGCA CAAGGACTTA ATTTCCAAA GATAATGCCCTG
2751 GCAGAAATTA GAGGATATAG AGTGATGCC GTCAAATGCC TTTAGACTCT
2801 GGCTTCCCTG GCTGTCTCAC ATCTCCCCCTA TTGGAGCTAA GTGTGGTGCT
2851 GTATTTAGCA GGGTATCTGG CAGATATTTT AAATTAATTG AAATATCACC
2901 CTAAATTTC AGATTCTGGT AAACCTGAAGT GAATTTTCAGA AATTATTGTA
2951 TTTATGTGTG TGCACATATG TGTGCAGGTA CCCACCGAAA TCTGCAGAGG
3001 CATCAGATGC CCCAGAGCTG GAACTGACAG TTGTGAGCCT GATATGAGTT
3051 CTGGGAATGA GCTCAGTCCT CTGGAAGAGC TGCAAGCACT ATTAAC TGCT

FIG. 3E

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3101 GAGCCATCTT TTCAGTCCCT CATGTATAGA TTAAAAAAA TTGGGGTTTG
3151 AAGATCCTCA TTTGTGAGAA ATTCCCTTCTT ACCTTTGCAA ACACTTTTTC
3201 TCATTTT TAG TATATGTATT CATATTTTAC TGTCTCATTT TCAATATATG
3251 TGGTCACAGT TTTTAAGTAT TTCTAAGGCA TAACAAGAT GTAATATTAA
3301 GAATAAATAA AAGAATAAAT CAATAATCCA GATGGTAGTG ACAGACACCT
3351 TTAATCCCAG TACTAAGGAG ACAGAGATAG GTAAATCTGT ATGAATTGA
3401 GACACGCC TG TTCTACAAAG AAATTTCAGG ACATCTAGGG GTATCCACAA
3451 AGAAACACTG TCTCAAAAAA TGCCAAACAA TCAAAAAA AAAA

FIG. 3F

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1 GTCAC TTTT AAGTATTAC CCAAGATATC TAAGGTTGCA GTTAGATAC
51 TCTATTACAT AGAGATCTTT AACATCTTT AAAAGGCTTT ATTTGTCTCT
101 GTTCACTTTA TTAATCCCGT TTATCCTTTG TCTATAGCAA TAGCTGGGTT
151 TTGGATTGA TCAGAGGAAA CAAAGTTCAG TCATTATCA CATGAGAGTT
201 GACAAGGTGT CTTTTTTTTT TCTCGTCACT GTACATAAAA AAATAAATAC
251 TACAAGAGGA AGGAACATTG TAGATGGAGA ATAGATAACT GACTAAAGG
301 GCTTTCCTTA GTCAAAAAGT TTAGGATCAA TATTATGAGT TTCTGATATT
351 CAATATTCA CCATGACTTA CAAGTACAGT GTTGTTTTT

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/07521

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.1, 69.5, 252.3, 320.1, 7.1, 7.2; 536/23.5; 530/350, 351; 514/2,8,12

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, BIOSIS, CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	TARTAGLIA et al. Identification and expression cloning of a Leptin Receptor, OB-R. Cell. 29 December 1995, Vol. 83, pages 1263-1271, see entire document.	1-5, 9-20 ----- 1-23
X --- Y	CHUA et al. Phenotypes of Mouse <i>diabetes</i> and Rat <i>fatty</i> due to mutations in the OB (Leptin) Receptor. Science. 16 February 1996, Vol. 271, see pages 994-996.	1-8, 12-20 ----- 1-23
X --- Y	CHEN et al. Evidence that the diabetes gene encodes the Leptin Receptor: Identification of a mutation in the Leptin Receptor gene in <i>db/db</i> mice. Cell. 09 February 1996, Vol. 84, pages 491-495, see entire document.	1-5, 12-20 ----- 1-23

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

•	Special categories of cited documents:	•	
• "A"	document defining the general state of the art which is not considered to be of particular relevance	• "T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
• "E"	earlier document published on or after the international filing date	• "X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
• "L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	• "Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
• "O"	document referring to an oral disclosure, use, exhibition or other means	• "A"	document member of the same patent family
• "P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 19 JUNE 1997	Date of mailing of the international search report T 1 JUL 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer GARNETTE D. DRAPER Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/US97/07521

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	CIOFFI et al. Novel B219/OB receptor isoforms: Possible role of leptin in hematopoiesis and reproduction. Nature Medicine. May 1996, Vol. 2, No. 5, pages 585-589, see entire document.	1-5, 9-20 ----- 1-23
X	WO 96/08510 A1 (PROGENITOR, INC.) 21 March 1996 (21.03.96), see the figures and claims.	1-23
X --- Y	LEE et al. Abnormal splicing of the leptin receptor in <i>diabetic</i> mice. Nature. 15 February 1996, Vol. 379, pages 632-635, see entire document.	1-5, 12-20 ----- 1-23
X	HODGSON J. Receptor screening and the search for new pharmaceuticals. Bio/Technology. September 1992, Vol. 10, pages 973-997, see entire document.	21-23
X	CA 2,104,996 A1 (BEHRINGWERKE AKTIENGESELLSCHAFT) 01 March 1994 (01.03.94), see the claims.	21-23

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/07521

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C12P 21/00; C12N 1/20, 15/00; G01N 33/53; C07H 21/04; C07K 1/00, 14/52; A61K 45/05, 38/19, 38/00

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

435/69.1, 69.5, 252.3, 320.1, 7.1, 7.2; 536/23.5; 530/350, 351; 514/2,8,12